

WHAT IS CLAIMED IS:

1 1. A method for screening a library of monomer domains or multimers
2 comprising monomer domains for binding affinity to multiple ligands, the method comprising
3 contacting a library of monomer domains or multimers of monomer domains
4 to multiple ligands; and
5 selecting monomer domains or multimers that bind to at least one of the
6 ligands.

1 2. The method of claim 1, comprising
2 i. contacting a library of monomer domains to multiple ligands;
3 ii. selecting monomer domains that bind to at least one of the ligands;
4 iii. linking the selected monomer domains to a library of monomer
5 domains to form a library of multimers, each comprising a selected monomer domain and a
6 second monomer domain;
7 iv. contacting the library of multimers to the multiple ligands to form a
8 plurality of complexes, each complex comprising a multimer and a ligand; and
9 v. selecting at least one complex.

1 3. The method of claim 2, the method further comprising
2 linking the multimers of the selected complexes to a library of monomer
3 domains or multimers to form a second library of multimers, each comprising a selected
4 multimer and at least a third monomer domain;
5 contacting the second library of multimers to the multiple ligands to form a
6 plurality of second complexes; and
7 selecting at least one second complex.

1 4. The method of claim 2, wherein the identity of the ligand and the
2 multimer is determined.

1 5. The method of claim 1, wherein a library of monomer domains is
2 contacted to multiple ligands.

1 6. The method of claim 1, wherein a library of multimers is contacted to
2 multiple ligands.

- 1 7. The method of claim 1, wherein the multiple ligands are in a mixture.
- 1 8. The method of claim 1, wherein the multiple ligands are in an array.
- 1 9. The method of claim 1, wherein the multiple ligands are in or on a cell
2 or tissue.
- 1 10. The method of claim 1, wherein the multiple ligands are immobilized
2 on a solid support.
- 1 11. The method of claim 1, wherein the ligands are polypeptides.
- 1 12. The method of claim 12, wherein the polypeptides are expressed on the
2 surface of phage.
- 1 13. The method of claim 1, wherein the monomer domain or multimer
2 library is expressed on the surface of phage.
- 1 14. The method of claim 1, wherein the monomer domain is a LDL
2 receptor type A monomer domain.
- 1 15. The method of claim 1, wherein the monomer domain is an EGF
2 monomer domain.
- 1 16. The method of claim 1, wherein the library of multimers is expressed
2 on the surface of phage to form library-expressing phage and the ligands are expressed on the
3 surface of phage to form ligand-expressing phage, and the method comprises
4 contacting library-expressing phage to the ligand-expressing phage to form
5 ligand-expressing phage/library-expressing phage pairs;
6 removing ligand-expressing phage that do not bind to library-expressing or
7 removing library-expressing phage that do not bind to ligand-expressing phage; and
8 selecting the ligand-expressing phage/library-expressing phage pairs.
- 1 17. The method of claim 16, further comprising isolating polynucleotides
2 from the phage pairs and amplifying the polynucleotides to produce a polynucleotide hybrid
3 comprising polynucleotides from the ligand-expressing phage and the library-expressing
4 phage.

1 18. The method of claim 17, comprising isolating polynucleotide hybrids
2 from a plurality of phage pairs, thereby forming a mixture of polynucleotide hybrids.

1 19. The method of claim 18, comprising
2 contacting the mixture of hybrid polynucleotides to a cDNA library under
3 conditions to allow for polynucleotide hybridization, thereby hybridizing a hybrid
4 polynucleotide to a cDNA in the cDNA library; and
5 determining the nucleotide sequence of the hybridized hybrid polynucleotide,
6 thereby identifying a monomer domain that specifically binds to the polypeptide encoded by
7 the cDNA.

1 20. The method of claim 1, wherein the monomer domain library is
2 expressed on the surface of phage to form library-expressing phage and the ligands are
3 expressed on the surface of phage to form ligand-expressing phage, and the selected
4 complexes comprise a library-expressing phage bound to a ligand-expressing phage and the
5 method comprises:

6 dividing the selected monomer domains or multimers into a first and a second
7 portion,

8 linking the monomer domains or multimers of the first portion to a solid
9 surface and contacting a phage-displayed ligand library to the monomer domains or
10 multimers of the first portion to identify target ligand phage that binds to a monomer domain
11 or multimer of the first portion;

12 infecting phage displaying the monomer domains or multimers of the second
13 portion into bacteria to express the phage; and

14 contacting the target ligand phage to the expressed phage to form phage pairs
15 comprised of a target ligand phage and a phage displaying a monomer domain or multimer.

1 21. The method of claim 20, further comprising isolating a polynucleotide
2 from each phage of the phage pair, thereby identifying a multimer or monomer domain that
3 binds to the ligand in the phage pair.

1 22. The method of claim 23, further comprising amplifying the
2 polynucleotides to produce a polynucleotide hybrid comprising polynucleotides from the
3 target ligand phage and the library phage.

1 23. The method of claim 20, comprising isolating and amplifying
2 polynucleotide hybrids from a plurality of phage pairs, thereby forming a mixture of
3 polynucleotide hybrids.

1 24. The method of claim 23, comprising
2 contacting the mixture of hybrid polynucleotides to a cDNA library under
3 conditions to allow for hybridization, thereby hybridizing a hybrid polynucleotide to a cDNA
4 in the cDNA library; and
5 determining the nucleotide sequence of the associated hybrid polynucleotide,
6 thereby identifying a monomer domain that specifically binds to the ligand encoded by the
7 cDNA associated cDNA.